BIOACCUMULATION AND EXPOSURE PATHWAYS OF SOIL CONTAMINANTS TO COTTON RATS ON PETROCHEMICAL SITES

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INTRODUCTION

This document consists of two chapters, each reporting separate studies conducted during my Master's program. Both chapters are presented in formats suitable for publication in professional journals.

CHAPTER I

SOIL AND VEGETATION FLUORIDE EXPOSURE PATHWAYS TO COTTON RATS ON A PETROCHEMICAL CONTAMINATED LANDFARM

Abstract

Fluoride (F) is ubiquitous in the environment occurring in uncontaminated soils (150 to 400 mg kg^{-1}) and vegetation (1 to 15 mg kg^{-1}). Petroleum refining produces oily-sludge wastes that may contain hydrofluoric acid. Landfarming is an economical means of disposing of oily sludges, but this process may result in contamination of soil and food chains in the ecosystem. Total F content of soils, vegetation, and cotton rats (*Sigmodon hispidus*) was measured in an ecosystem contaminated from the disposal of petrochemical wastes by landfarming. A potentially bioavailable form of F was also determined by HCl extraction of soils and vegetation. Cotton rats from the landfarm study site were examined for prevalence of dental lesions indicative of fluorosis. A model was constructed to evaluate two exposure pathways: dietary ingestion of vegetation and non-dietary ingestion of soil. Mean bone F (1515 mg kg^{-1}) and mean total soil F (1954 mg kg^{-1}) from the landfarm site were more than ten-fold greater than F levels in bone (121 mg kg^{-1}) and mean total soil F (121 mg kg^{-1}) at a matched reference site. The HCl-extractable form of F in soil was elevated (326 mg kg^{-1}) compared to the reference site (2.3 mg kg^{-1}), suggesting high levels of potentially bioavailable F existed. About 80% of the cotton rats collected from the landfarm had dental fluorosis. Total F in johnsongrass *(Sorghum halapenes)* on the landfarm (9.5 mg

kg^{-1}) was slightly elevated compared to the matched reference site (3.8 mg kg^{-1}) in summer. Total F in brome grass (*Bromus spp.)* on the landfarm site (33.4 mg kg^{-1}) was significantly higher than on the reference site (12.4 mg kg^{-1}) in winter. During winter, the dietary pathway consisted of 78.9% of the bioavailable exposure of the two pathways. However, in the summer, the non-dietary pathway consisted of 87.9% of the bioavailable exposure of the two pathways. Fluoride accumulation in the soil from landfarming of petroleum wastes may pose a risk to the health of terrestrial vertebrates. In addition to monitoring of total petroleum hydrocarbons, land application of petrochemical wastes should consider F and other inorganic contaminant loadings to the soil system.

Introduction

Fluoride (F) is ubiquitous in the environment occuring in uncontaminated soils (150 to 400 mg kg^{-1}) and vegetation (1 to 15 mg kg^{-1}) (Kabata-Pendias and Pendias, 1984). Fluoride is also used in a variety of industrial processes including electroplating, wood preservation, paper production, and the production of petroleum products. Petroleum refining produces a variety of wastes including hydrofluoric acid which is used as a catalyst in gasoline production. Waste hydrofluoric acid is neutralized and often disposed of in waste-sludge pits (Gary and Handwerk 1984). Landfarming is frequently used by petroleum refineries as an economical means of disposing of oily sludges that may contain F (Golueke and Diaz 1989). Landfarming involves the application of waste to soil followed by fertilizer addition and tillage to promote aerobic decomposition (Baker 1994). Because F does not biodegrade, elevated levels of F may occur on landfarmed areas.

Small amounts of F are often added to tooth paste or municipal water systems to prevent dental caries in humans (Horowitz 1980). However, excessive F exposure may cause problems in humans, domestic stock, and wildlife. Shupe et al. (1972) noted dental lesions in livestock and showed that exposure to excessive levels of F could cripple domestic livestock. Exposure to elevated F may result in dental and skeletal changes (fluorosis) in certain species of wildlife (Boulton et al. 1994a, Kierdorf et al. 1995, Vikoren and Stuve 1996).

Most studies on wildlife exposed to excessive F contamination have focused on aluminum mine waste areas or areas adjacent to aluminum smelters where aerial deposition has resulted in F contamination of soils and vegetation. Recently, Paranjpe et al. (1994) reported a high prevalence of fluorosis in cotton rats (*Sigmodon hispidus*) residing at an abandoned petrochemical refinery in Cyril, Oklahoma. They reported macroscopic and microscopic lesions in both upper and lower incisors of the cotton rats captured from the refinery. Mean bone fluoride of the cotton rats captured from the refinery site of 1657 mg kg^{-1} was significantly higher than rats captured at reference sites (mean of 192 mg kg^{-1}) Although landfarmed waste was suspected as the source of F exposure, Paranjpe et al. (1994) did not document the F source or establish a relationship between soil F and fluorosis.

Few studies have documented exposure pathways for F to wildlife. Evaluation of exposure pathways and concentrations of F in terrestrial and aquatic organisms is extremely important to assess the importance of bioaccumulation as a possible route of human exposure (U.S. Department of Human and Health Services 1993). Soil as well as vegetation has been implicated as an exposure source, but very little information on the relative importance of each pathway is available. Davison (1987) found most studies reported vegetation and wildlife tissue F, but few studies reported soil F. Even fewer studies have focused on detailing pathways or quantitative measurements of transport.

Cotton rats are indigenous to Oklahoma and serve a critical functional role in terrestrial ecosystem food chains. Because cotton rats have constantly erupting incisors, they may be a useful biomonitor for F contamination in ecosystems. The objectives of this study were (1) to determine the relationship between bone F, soil F, and fluorosis in cotton rats residing in an abandoned landfarm site where petrochemical wastes were applied to soil, and (2) to document the relative importance for two F-exposure pathways for cotton rats on a landfarm site.

Methods and Materials

Petrochemical Contaminated Landfarm

The Oklahoma Refining Company is located in Cyril, Caddo County, Oklahoma. The refinery was active until 1984 when bankruptcy was declared and the facility was closed and abandoned. The refinery is approximately 63 ha in size and is composed of the main processing facility, earthen ponds, storage pits, above ground storage tanks, and a 3.4-ha landfarm site where oily sludges had been applied. Cotton rats, soils, and vegetation were collected from the landfarm and from a matched reference site adjacent to the facility. A reference site that showed no visible evidence of petrochemical contamination and was not on the refinery property was selected for comparison. Both sites were similar in vegetative composition and contained adequate populations of cotton rats for trapping.

Collection of Animals and Preparation of Bones

A total of 48 adult cotton rats were collected from a 160 m x 40 m grid on the landfarm and the reference site during winter and summer. Cotton rats were captured with Sherman live traps placed on a 10-m spacing and baited with rolled oats. Following capture, the cotton rats were housed overnight and sacrificed the following morning by exsanguination. The two humeri were removed from each cotton rat, cleaned of excess tissue with a scalpel, dried by lyophilization, weighed, and placed in petroleum ether for 96 h with daily changes to eliminate fat. At time of sacrifice, cotton rat skulls were removed and formalin fixed for later evaluation of incisors for presence of dental fluorosis.

Bone Fluoride Analysis

Prepared bones were acid digested by a method adapted from Andrews et al. (1989). Each pair of bones (≈100 mg) was placed in a 25 ml Teflon beaker and refluxed on a hotplate at 95^0 C with 5.0 ml of trace metal grade HNO_3 for 1.0 h. The acid digest was diluted to volume with deionized distilled water in a 10 ml volumetric flask. A 1.0 ml aliquot of the diluted digest was further diluted with deionized distilled water to a volume of 5.0 ml and subsequently combined with an equal volume of TISAB II buffer to adjust ionic strength and inhibit complexation of F by Fe and Al interferrants (Frankenberger et al. 1996, Orion 1996). Solution pH was checked and adjusted to 5.0-5.5 by adding ≈500 µl of 10 M NaOH. Calibration standards were prepared in a similar manner from Fisher-certified 100 mg L^{-1}, and fluoride concentration was determined with an Orion

combination fluoride ion-selective electrode and reported as mg kg^{-1} on a freeze-dried basis. Blanks, standard reference material (NIST bone meal srm 1486), and spike recoveries were used for quality assurance.

Scoring of Teeth for Dental Lesions

Scoring of incisors was performed to document gross morphological lesions commonly referred to as fluorosis using a system previously described for mammals (Boulton et al. 1994a, Boulton et al. 1995, Shupe et al. 1972) (Table 1). All cotton rats were assigned a random number to prevent bias and were scored by two different analysts for confirmation.

Collection and Analysis of Soils

Surface soils (<2 cm) were collected from the trapping grid on the landfarm site which consisted of four lines with sixteen traps per line. Soil samples were collected from every other trap for a total of 32 composite soil samples. Six soil subsamples were mixed to form each composite soil sample. Because there was less variability in soil content of fluoride, only two composite soil samples composed of six subsamples each were collected from the reference site. All soils were stored and transported in sealed acid-washed glass jars. Soils were air-dried and sieved to pass a 2 mm screen prior to analysis.

Some chemical and physical properties (pH, organic carbon, electrical conductivity, and texture) were measured on the soil samples. Soil pH (7.5) was determined in a 1:2 soil:0.01 M $CaCl_2$ suspension (Thomas 1996). Electrical

conductivity (241 $\mu S\ cm^{-1}$) was measured in a 1:5 soil:deionized water extract (Rhoades 1996). Soil organic carbon (3.2%) was determined by an acid dichromate wet digestion method (Yeoman's and Bremner 1988). Soil texture (loam) was determined by the hydrometer method of Gee and Bauder (1986).

Three procedures were used to fractionate fluoride in contaminated soils into (1) readily soluble F, (2) potentially bioavailable F, and (3) total F. Readily soluble F was measured by placing 2 g of soil in a 40 ml plastic centrifuge tube, shaking with 10 ml of a 0.01 M KNO_3 solution for 1.0 h, and vacuum filtering through a 0.45 μm Supor membrane filter. Five ml of the extract was combined with 5.0 ml of TISAB II. Fluoride was then determined with an Orion combination ion-selective electrode and reported as mg kg^{-1} on a dry weight basis. Blanks and spike recoveries were used for quality assurance.

A weak acid extraction (0.03 M HCl, pH 1.5) was used to simulate stomach conditions and extract potentially bioavailable F as described by Walton (1987). Small mammals maintain a constant pH in their stomach by releasing more gastric juices to overcome the buffering effect of ingested soil. Soil (1 g) was extracted with 20 mL of dilute HCl by reciprocal shaking for 30 min. A 20:1 HCl solution:soil ratio was chosen to overcome the buffering effect of ingested soil and changes in extraction pH between samples. Solution pH was unchanged during the extraction showing that the extraction was not affected by the buffering capacity of the soil. Fluoride in acid extracts was determined by potentiometric methods using a F electrode as described for readily soluble F.

Because acid extractions of soils result in low recoveries of total fluoride due to the presence of non-acid labile fluorides (Hall 1968, Cooke et al. 1976, Andrews et al. 1989), fusion techniques are required to accurately measure total soil F (Venkateswarlu 1983). The method of McQuaker and Gurney (1977) was employed to decompose the soils by fusion with NaOH followed by fluoride determination using an ion-selective electrode. In this method, 0.5 g of soil was placed in 100 ml nickel crucibles and slightly moistened with deionized distilled water. Concentrated NaOH (19 M) was added to the sample and fused in a muffle furnace at 600^0 C. The fusion cake was dissolved in deionized distilled water and neutralized with concentrated HCl to pH 8-9. The cooled sample was then transferred to a 100 ml volumetric flask, diluted to volume, and filtered through a 0.45 Supor membrane filter. Sample digest (5.0 ml) was combined with 5.0 ml of TISAB II and fluoride was determined with an Orion combination ion-selective electrode and reported as mg kg^{-1} on a dry weight basis.

Collection and Analysis of Vegetation

Recent work on the microscopic examination of stomach contents has shown that the diet of cotton rats in this area of Oklahoma is seasonally dependent and that their predominant food source is johnsongrass (*Sorghum halapenes*) in the summer and brome grass (*Bromus spp.)* in winter (Schetter et al. 1998). Thus, johnsongrass was collected during August and brome grass was collected during January from the landfarm site for fluoride analysis. Johnsongrass vegetation was collected from the same sampling point as the soil

samples at every other trap. Mature johnsongrass was separated into its primary components of leaves, stems, and seeds prior to analysis. A total of 32 brome grass samples were obtained in the immature growth stage so the whole plant was analyzed. Two johnsongrass and two brome grass samples from the reference site were obtained from the same sampling point as the reference soils. All vegetation samples were oven-dried at 60^0 C for 96 h and ground (<2 mm) in a Wiley mill. Finally, plant samples were ground in a Udy mill (<500 μm) to obtain a homogenous sample for plant analysis (Jones and Case 1990).

Two different procedures, used to extract vegetation, were total fluoride content and a potentially bioavailable fluoride fraction. Total content of fluoride was determined by acid digestion. Plant tissue (100 mg) was placed in 25 ml Teflon beakers with 2.0 ml of trace metal grade HNO_3 and was refluxed on a hotplate at 95^0 C for 1.0 h. Acid digests were analyzed by potentiometric methods using a F electrode as described for bone samples. The potentially bioavailable fraction of fluoride in vegetation was measured by extraction of vegetation with a weak hydrochloric acid solution to simulate gastric juices (Walton 1987, Boulton et al. 1994a). Vegetation (1 g) was placed in a 40 ml plastic centrifuge tube and shaken with 20.0 ml HCl (pH 1.5) for 30 min. The HCl extract was analyzed by potentiometric methods using a F electrode as described for soil samples.

Exposure Pathway Model

Two potential pathways of exposure to F, a dietary pathway and a non-dietary pathway, were evaluated in this study. The dietary pathway examined fluoride ingested during normal feeding behavior (primarily vegetative fluoride with some fluoride from possible soil contamination of vegetation). The non-dietary pathway included any incidental ingestion of soil from such activities as burrowing and grooming.

Several assumptions were used in the assessment of exposure pathways. Because adult cotton rats weigh between 100-200 g (Cameron and Spencer 1981), an average adult weight of 150 g was used in all calculations. Dietary ingestion rates for cotton rats averages 0.10 g of dry mass per gram of live body mass per day (Randolph et al. 1995). Thus, a mean dietary consumption of 15 g dry weight day^{-1} rat^{-1} was used to assess exposure pathways. Garten (1980) found that 2.8% of the dry matter in the cotton rat's stomach and intestines was derived from ingested soil. Therefore, the non-dietary incidental ingestion used to assess exposure pathways was 2.8% of 15 g day^{-1} or 0.42 g soil day^{-1} rat^{-1}. Dominant dietary vegetation considered for pathway analyses was johnsongrass in summer and brome grass in winter.(Schetter et al. 1998). Cotton rats ingest ≈21.5% johnsongrass in the form of leaves and stems with the remaining 78.5% as seeds in summer (Schetter et al. 1998). Thus, we estimated that cotton rats ingest approximately 3.13 g of leaves and stems, 11.44 g of seeds, and 0.42 g of soil per day in summer. Daily winter diet was assumed to be 14.58 g brome grass and 0.42 g of soil. The potentially bioavailable F fraction of soil and plant

material, which measures the most likely forms of F dissolved in the gastrointestinal tract, was used to quantify fluoride exposure to the cotton rat in this model.

Results and Discussion

Dental Lesions

Cotton rats are herbivorous rodents which depend on their teeth to harvest and masticate vegetation prior to digestion. Gross morphological lesions in teeth of small mammals may serve as an indicator of fluorosis and may impair feeding behavior (Cooke et al. 1996). In fact, the continuously growing incisor of the rat has been commonly used as a model for laboratory fluoride experiments (Fejerskov et al. 1983). A strong relationship exists between severity of dental fluorosis and bone F of cotton rats collected from the landfarm and reference sites (Figure 1). In a previous study at the Cyril refinery, cotton rats were captured and examined for incisor lesions (Paranjpe et al. 1994). This work showed that 94 of 97 cotton rats captured from the refinery site had dental fluorsis; however, cotton rats from the reference site all displayed normal incisors. Mean bone F of 11 cotton rats of 1657 mg kg^{-1} from the refinery site was higher than mean bone F of 11 cotton rats of 192 mg kg^{-1} from the reference site (Paranjpe et al. 1994). These results were similar to those from our study where ≈80% of the cotton rats collected from the landfarm site had severity scores >3 compared to those from the reference site (severity score of 0-1). Cotton rats collected from the landfarm displayed the classic symptoms of fluorosis.

Dental lesions occur in different species of small mammals collected from areas contaminated with fluoride. Walton (1986a) found elevated bone F and dental lesions in moles (*Talpa europaea*) and shrews (*Sorex araneus*) captured from a site adjacent to an aluminum smelter in Europe. In another study at the same site, Walton (1986b) reported voles (*Microtus agretis*) and wood mice (*Apodemus sylvaticus*) displayed dental lesions and a mean bone F of 7148 mg kg^{-1} for voles and 8430 mg kg^{-1} for wood mice. Other studies have reported a relationship between severity of dental fluorosis and bone F in red deer (*Cervus elaphus*) (Kierdorf et al. 1995) and other rodents (Boulton et al. 1994a). This relationship has also been demonstrated by Boulton et al. (1995) in the laboratory where wild species of rodents were dosed with several concentrations of sodium fluoride in their drinking water. However, some studies have found elevated bone F did not cause dental lesions. Andrews et al. (1982) found elevated levels of bone F (554 to 1,283 mg kg^{-1}) without dental fluorosis in voles (*Microtus agretis*) and shrews (*Sorex araneus*) collected from an area to which waste minerals generated during the separation of fluorspar had been applied. Wright and Davison (1978) noted a lack of dental lesions in an area adjacent to a contaminated tailings dam. Mice (*Apodemus sylvaticus*) and voles (*Microtus agretis*) collected from this area had mean bone F ranging from 379 to 1077 mg kg^{-1}. These studies suggest that certain species may be more tolerant to fluoride and have different threshold limits in their skeletal systems before any visible impact is seen.

Bone Fluoride and Soil Fluoride

Elevated levels of fluoride (F) in cotton rat bones and soil ($p < 0.05$) were associated with the landfarm site (Table 2). Bone F and total soil F from the landfarm were more than 10-fold greater than those associated with the reference site. Bone F in rats from both the landfarm and the reference site contained levels that were similar to the levels previously found at the Cyril refinery (Paranjpe et al. 1994). Bone F of cotton rats collected on the reference site was also similar to background levels of other small mammal studies. Kay et al. (1975) found mean bone F concentrations of 133 to 144 mg kg^{-1} for three species of rodents (deer mice and two species of meadow voles) on an uncontaminated site in Montana. Total soil F content of the reference site was similar to total soil F of uncontaminated sites which ranges from 150 to 400 mg kg^{-1} (Kabata-Pendias and Pendias, 1984). Schroder (1998) reported a strong relationship between bone F and soil F for cotton rats collected from 11 petrochemical contaminated sites in Oklahoma (Figure 2).

Andrews et al. (1982) found fluoride contamination of soil (8905 mg kg^{-1}) and elevated bone F (554 to 1283 mg kg^{-1}) in voles (*Microtus agretis*) and shrews (*Sorex araneus*) collected from an area to which waste minerals generated during the separation of fluorspar had been applied. Wright and Davison (1978) reported mean total soil F as being 7050 mg kg^{-1} from an area adjacent to a contaminated tailings dam where F concentration was >100,000 mg kg^{-1} and mean bone F for field mice (*Apodemus sylvaticus*) and field voles (*Microtus agretis*) ranged from 379 to 1077 mg kg^{-1}. Field voles (*Microtus agretis*) caught

on a reclaimed tailings dam with soil F >100,000 mg kg^{-1} had dental lesions and elevated bone F of 1106 mg kg^{-1} (Andrews et al. 1989). Similarly, elevated bone F in cotton rats from petrochemical sites occurred in this study but soil F levels in this study were much lower than the degree of contamination of soils in studies associated with aluminum smelting and tailings.

Total soil F may not be a good indicator of the amount of F bioavailable to plants or mammals because many soil forms are very insoluble and are unlikely to dissolve in soil solution or the gastrointestinal tract. Plant available "readily-soluble F" and "HCl extractable F" which is potentially bioavailable to mammals were measured. Schroder (1998) reported a strong relationship between the HCl extractable F and bone F ($p < 0.05$) (Figure 3). Readily soluble F at the contaminated landfarm site was higher than typical water soluble F for uncontaminated sites of 0.02 mg kg^{-1} (Frankenberger et al. 1996) suggesting more plant available F in the contaminated soil (Table 2). Most soil F was not dissolved by the pH 1.5 HCl extract used in this study (Table 2). However, HCl extractable F was elevated suggesting high potentially bioavailable F to cotton rats.

Vegetation Fluoride

Mean values show that total F for johnsongrass (leaves and stems) and brome grass from the landfarm was elevated ($p < 0.05$; Table 3). A few brome grass samples had very high total F (> 50 mg kg^{-1}), which resulted in a large range of plant F.

The potentially bioavailable extraction for brome grass was higher ($p < 0.05$) than the reference site. The bioavailable F for johnsongrass (leaves and stems) was slightly elevated on the landfarms compared to the reference site ($p < 0.05$). Fluoride is not an essential element for plants, and large amounts may impair plant growth. The F content of plants from uncontaminated areas typically ranges from 1 to 15 mg kg^{-1} and rarely exceeds 30 mg kg^{-1}. However, on highly contaminated sites, plants can accumulate F from soils or by aerial deposition (Kabata-Pendias and Pendias 1994). Cooke et al. (1976) observed that plants grown in soils containing 27,000 to 174,000 mg kg^{-1} accumulated F of 609 to 5856 mg kg^{-1} (washed vegetation). Andrews et al. (1989) found elevated levels of F (300 to 1000 mg kg^{-1}) in unwashed vegetation collected from a highly contaminated reclaimed fluorspar tailings dam with soil F exceeding 100,000 mg kg^{-1}. Voles collected from the site displayed dental lesions. Boulton et al. (1994b) examined unwashed vegetation collected from three different fluoride contaminated sites and measured both total F and hydrochloric acid-extractable F. Total vegetation F for his reference site was 20 mg kg^{-1} and the contaminated sites ranged from 80 to 549 mg kg^{-1}. Field and bank voles collected from all the sites had dental lesions. Cooke et al. (1996) state that vegetation containing greater than 40 mg kg^{-1} total F may have a significant impact on wildlife. Results from this study indicate that the brome grass collected from the landfarm is close to this critical value and may have a significant impact on cotton rats.

Exposure Model

A model was constructed to estimate the relative importance of two fluoride exposure pathways. The non-dietary pathway examined incidental ingestion of soil, whereas the dietary pathway examined exposure due to ingestion of vegetation. The dietary pathway consisted of 78.8% of the bioavailable exposure in the winter (Table 4). The dietary pathway bioavailable F was greater than the non-dietary pathway suggesting consumption of vegetation was more important than incidental ingestion of soil. However, in the summer, the non-dietary pathway consisted of 87.9% of bioavailable F exposure suggesting incidental ingestion of soil was the primary pathway for exposure. Because vegetation was unwashed, this seasonal difference may be the result of soil contamination of brome grass samples. Schupe et al. (1972) state that most plants do not translocate dangerous levels of F, but plants with high levels of F may be contaminated with soil from rain splash or dust storms. Healy (1973) found that field-collected pasture herbage was contaminated with 25-35% soil. Boulton et al. (1994b) reported a diet of unwashed vegetation (100 mg F kg^{-1}; 25 mg F/kg/day) from a F contaminated site resulted in fluorosis and elevated bone F in voles under laboratory conditions. Field voles collected from the same site as the contaminated vegetation had dental lesions and elevated bone F. Regardless of whether bioavailable F was from vegetation or from soil contamination of the unwashed vegetation, results from Boulton et al. (1994b) suggest the dietary pathway can cause fluorosis. Results from this study suggest that contaminated soil is the predominant source of potentially bioavailable F in

both dietary and non-dietary pathways.

Conclusion

Soil and bone F for cotton rats collected from the Cyril landfarm site were highly elevated as compared to the matched reference site. In addition, the majority of cotton rats captured on the landfarm site had dental lesions characteristic of fluorosis. There was a good relationship between an incisor score and bone F for the cotton rats from the Cyril landfarm. Mean soil F was related to mean bone F for cotton rats and soils collected from Cyril. Brome grass consumption is a significant fluoride exposure pathway during the winter. However, during the summer, incidental ingestion of soil is more important than consumption of vegetation for cotton rats on the landfarm site.

Landfarming is extensively used by the petroleum industry for disposal of waste. However, many inorganics including fluoride and metals do not biodegrade and tend to accumulate when landfarmed. Thus, the practice of landfarming may pose a threat to the health of terrestrial organisms. The degree of risk is related to the inorganic F content of the waste and the potential exposure pathways. We suggest that not all petrochemical waste may be safely landfarmed when the overall health of the ecosystem is considered. In addition to monitoring of total petroleum hydrocarbons, waste should be analyzed for inorganic contaminants and land application rates should be based on inorganic contaminant loadings to the soil system. Waste that contains excessive levels of inorganic contaminants may not be suitable for land application.

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Table 1. Scoring system for assessing severity of fluoride-induced lesions in incisors of cotton rats

Score	Incisor Characteristics
0	Normal: smooth, glossy deep yellow-orange
1	Slight striation or mottling in lower incisor
2	Definite mottling or striation (white chalky) in lower incisors
3	White chalky lower incisors; slight mottling in upper incisors
4	White chalky lower incisors; definite striation (or mottling) in upper incisor
5	White chalky lower and upper incisors

Table 2. Statistical summary of bone fluoride and forms of soil fluoride from contaminated landfarm and reference site. (all measurements are in mg kg^{-1})

			Soil F		
Site	Parameter	Bone Total F	Readily Soluble[a]	Bioavailable[b]	Total[c]
Landfarm	Mean	1,515*	4.8*	326*	1,954*
	St Dev	777	3.0	173	1,871
	Range	272-3,790	1.2-12.6	21.9-699	38.1-5,871
Reference	Mean	121	0.2	2.3	121
	St Dev	42.6	0.0	0.4	4.3
	Range	46-218	0.2-0.2	2.0-2.5	117-124

[a]0.01 M KNO_3 extract
[b]Bioavailable F (1.5 pH HCl extraction)
[c]NaOH fusion
Asterisks (*) indicate values that are greater than reference site ($p < 0.05$).

Table 3. Statistical summary of vegetation fluoride from contaminated landfarm and reference site. (all measurements are in mg kg^{-1})

		Johnsongrass					
		Leaves And Stems		Seeds		Brome Grass	
Site	Parameter	Bio F [a]	Total F	Bio F	Total F	Bio F	Total F
Landfarm							
	Mean	1.3*	9.5*	1.3	8.3	35.0*	33.5*
	St Dev	0.7	5.0	0.6	4.8	39.4	33.8
	Range	0.6-4.9	3.5-23.0	0.8-4.0	3.9-24.4	3.7-140	5.2-129
Reference							
	Mean	0.9	3.8	0.9	5.8	1.6	12.4
	St Dev	0.1	1.5	0.1	2.9	0.2	1.1
	Range	0.8-1.0	2.5-5.5	0.8-1.0	3.7-7 8	1.4-1.7	11.6-13.1

[a] Bioavailable F (1.5 pH HCl extraction)

Asterisks (*) indicate values that are greater than reference site ($p < 0.05$).

Table 4. Bioavailable fluoride exposure model summarizing non-dietary (soil) and dietary (plant) pathways for cotton rats from a contaminated landfarm.

Season	Exposure Pathway	Bioavailable F^- ($\mu g\ day^{-1}$)	% Bioavailable F^-
Winter	non-dietary[a]	137	21.2
	dietary[b]	510	78.8
	non-dietary + dietary	647	100.0
Summer	non-dietary	137	87.9
	dietary	18.9	12.1
	leaves and stems	4.07	2.61
	seeds	14.9	9.54
	non-dietary + dietary	156	100.0

[a]Pathway includes incidental ingestion of soil by inhalation, grooming, or burrowing.
[b]Pathway estimates consumption of plants through normal feeding behavior.

Fig. 1. Bone incisor rating vs. bone fluoride for cotton rats collected from petrochemical contaminated landfarm

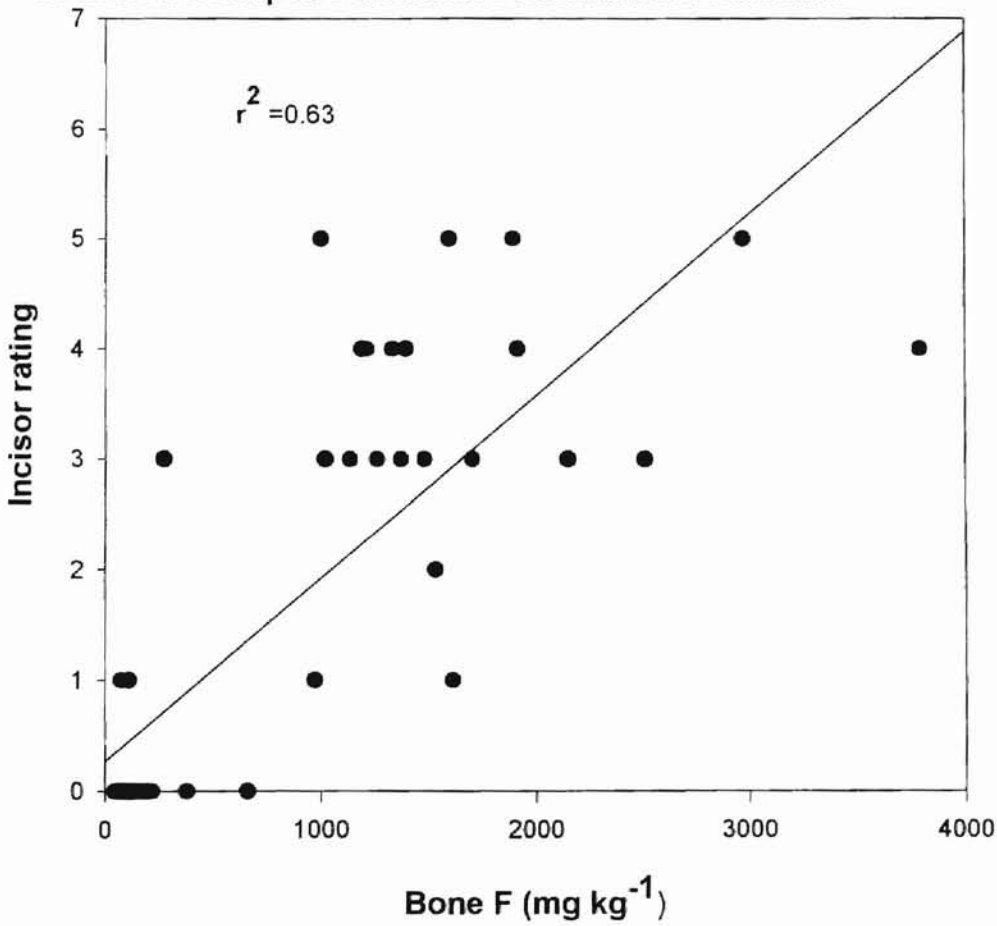


Fig. 2. Mean bone fluoride vs. mean total soil fluoride for petrochemical contaminated sites

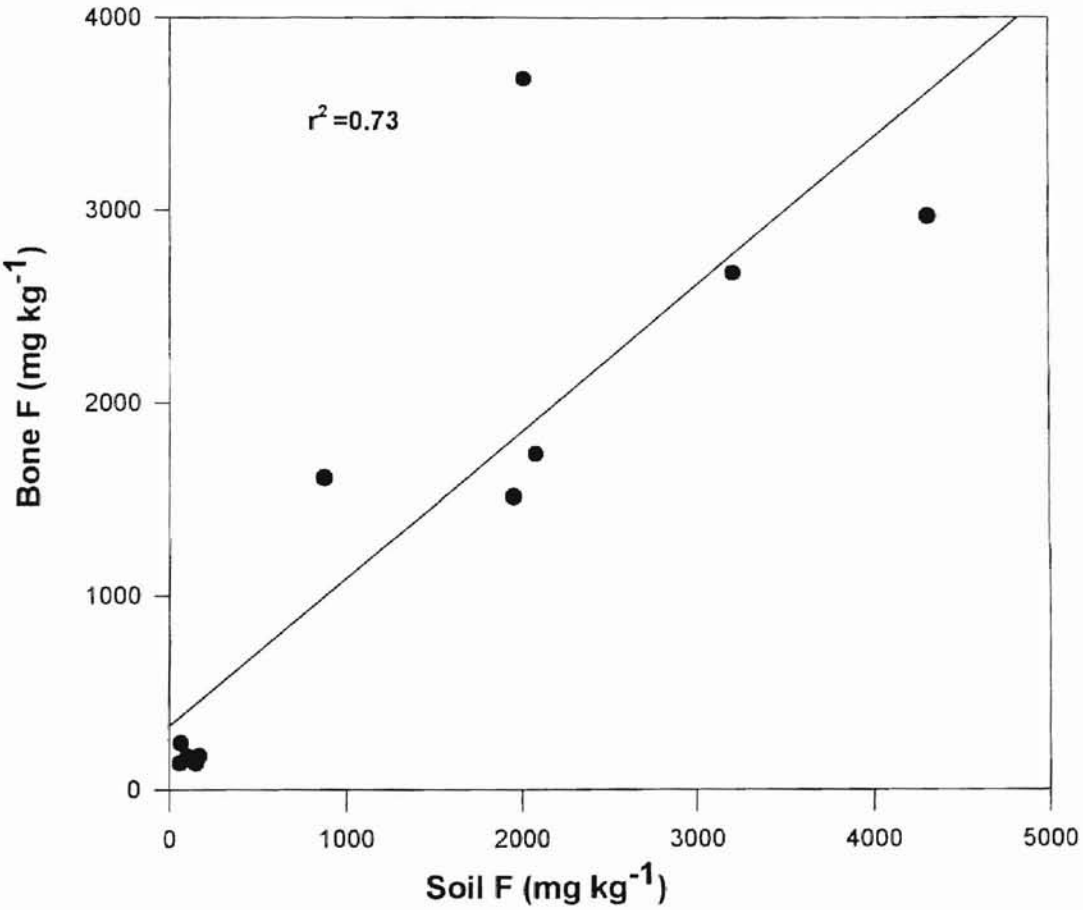
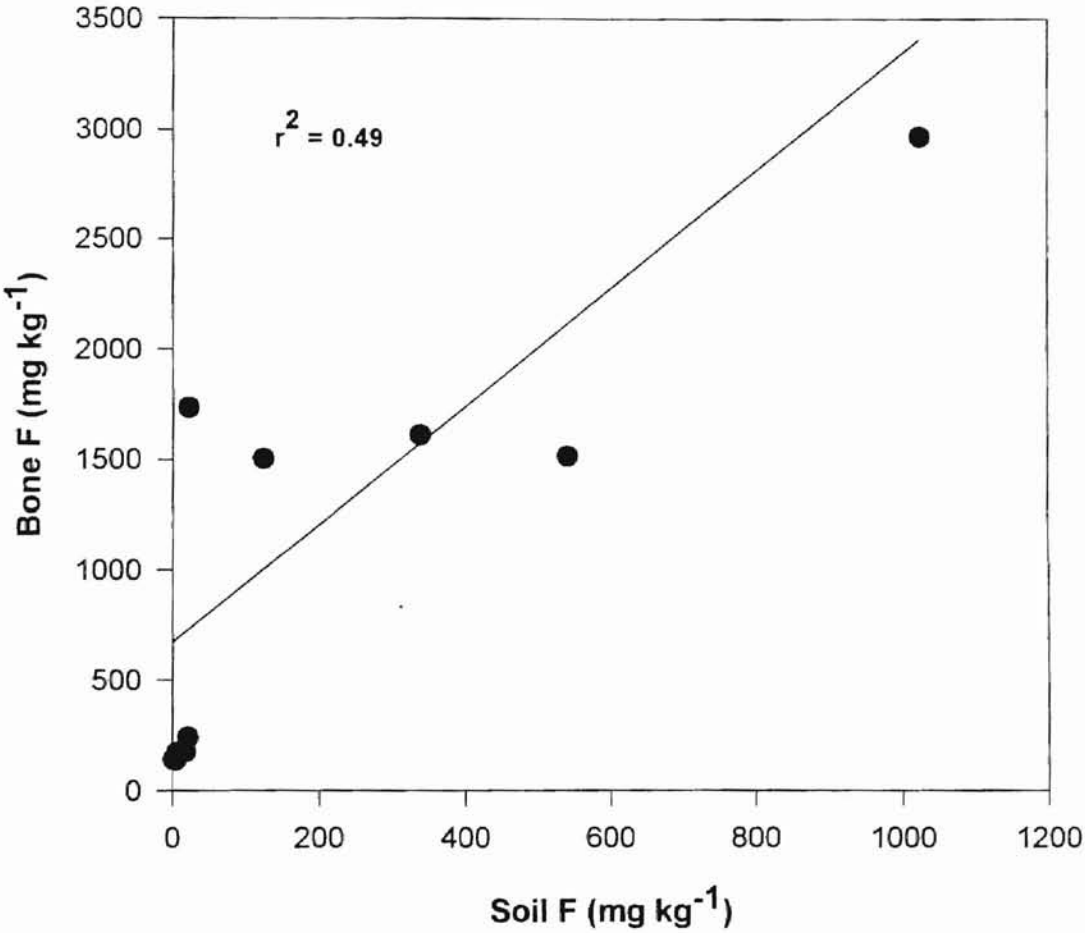


Fig. 3. Mean bone fluoride vs. mean HCl extractable soil fluoride for petrochemical contaminated sites



Chapter II

SOIL CONTAMINATION AND BIOACCUMULATION OF METALS AND FLUORIDE IN COTTON RATS FROM PETROCHEMICAL SITES

Abstract

Petrochemical wastes usually contain inorganic chemicals (i.e. metals and fluoride) which may accumulate and pose ecological risks to wildlife. Small mammals have been used as indicators of contamination and transfer between trophic levels in small mammals has been shown for elements such as cadmium, lead, and fluoride. The objectives of this study were (1) to evaluate the extent of soil contamination with metals and fluoride on petrochemical sites (2) to determine bioaccumulation of metals and fluoride in cotton rats residing on petrochemical sites and (3) to determine the relationship between metal and fluoride concentrations in contaminated soil and in bone of cotton rats collected from petrochemical sites. Cotton rats (*Sigmodon hispidus*) and soils were collected from 12 petrochemical sites (landfarms, pond burms, and tar pits) and matched reference sites. Soils and cotton rat bones were analyzed for metals and fluoride. Soils of petrochemical sites were contaminated with Cd, Cr, Cu, Ni, Pb, Sr, Ti, V, and Zn. Metal contamination was randomly distributed among landfarms, pond burms, and tar pits. Fluoride in soil was elevated (10- to 60-fold greater) on the petrochemical sites as compared to the reference sites and was

more prevalent on landfarms. Fluoride and Pb were also elevated in bone tissue of cotton rats collected from the petrochemical sites as compared to the reference site. Lead levels in bone of 21.5 mg kg^{-1} were higher in cotton rats collected from the petrochemical sites in winter as compared to Pb of 10.0 mg kg^{-1} in bone of animals collected during the summer. Most cotton rats (80%) collected from seven petrochemical sites with elevated levels of soil and bone fluoride had dental fluorosis. The prevalence of dental fluorosis was 50% higher in winter than in summer animals. Although elevated levels of metal were found in both soils and cotton rats from the petrochemical sites, there was not a strong relationship between metal content of bone and soil metal concentrations. However, there was a strong relationship ($r = 0.85$) between bone fluoride and total content of fluoride in soil. To prevent accumulation of contaminants in cotton rats, land application of petrochemical wastes should be based on inorganic contaminants. Wastes that contain excessive levels of inorganic contaminants may not be suitable for land application.

Introduction

The petrochemical refining industry utilizes a variety of chemicals in the processing of crude oil and produces a larger quantity of hazardous or non-hazardous waste. In 1991, the petroleum industry produced approximately 270 billion pounds of hazardous waste (Bass et al. 1995). Much of this waste is usually disposed or is stored on site. Disposal on site is usually by landfarming of the waste by application of waste to soil followed by fertilizer addition and tillage to promote biodegradation (Baker 1994). Storage on site involves the construction of a sludge pit that is enclosed by a pond burm. Excessive rainfall may overflow the sludge pit and deposit wastes on adjacent areas to the pit (a tar pit). There are many unique areas present on petrochemical sites that represent different exposures to wildlife. Many of these areas are heavily vegetated and may support populations of small mammals and other vertebrates (McMurry 1993).

Petrochemical wastes usually contain inorganic chemicals (i.e. metals and fluoride) which do not biodegrade. These inorganics may accumulate and pose ecological risks to wildlife present on these areas. Metals that are present in petrochemical wastes and that have been shown to possess immunotoxic properties include lead, chromium, and vanadium (IPCS 1986). Small mammals may be exposed to contaminants through a number of processes which may include incidental ingestion of soil, ingestion of water, and ingestion of food sources (both plant and animal) that contain the contaminant. Small mammals have been used as indicators of contamination with residues being determined in

whole body residues or in specific organs. Uptake of contaminants and transfer between trophic levels in small mammals for elements such as cadmium, lead, and fluoride has been shown to occur on contaminated sites (Andrews et al. 1989a,b; Hunter 1987). Small mammals have also been successfully used to document exposure and toxicity of both fluoride and metals (Johnson et al. 1978, Roberts et al. 1978, Walton 1986, Boulton et al. 1994). Cotton rats (*Sigmodon hispidus*) are indigenous to Oklahoma and serve a critical functional role in terrestrial food chains. They have also been used as successful biomonitors for both metals and fluoride (Paranjpe et al. 1994, McMurry et al. 1995, Schroder, 1998). Few studies have evaluated contaminants on petrochemical sites in both soil and in wildlife. The objectives of this study were (1) to evaluate the extent of soil contamination with metals and fluoride on petrochemical sites (2) to determine bioaccumulation of metals and fluoride in cotton rats residing on petrochemical sites and (3) to determine the relationship between metal and fluoride concentrations in contaminated soil and in bone of cotton rats collected from petrochemical sites.

Methods and Materials

Study Sites

Cotton rats and soils were collected and analyzed over a three-year period from 12 petrochemical sites and matched reference sites. Four different sites were sampled each year. Sites were classified into three categories: landfarm, pond burm, or tar pit based on past management practices. Sites A through E

were landfarms where oily-sludges had been applied for disposal purposes. Sites F through H were pond burms constructed to contain oil sludges and sites J through L were tar pits that were subjected to periodical overflow of material from oil sludge pits (Table 1). All petrochemical sites were privately owned and contained adequate populations of cotton rats for trapping. Reference sites were chosen in the vicinity of the petrochemical sites and were selected to match petrochemical site vegetation. Reference sites showed no visible evidence of petrochemical contamination and contained adequate populations of cotton rats

Collection and Analysis of Soils

Surface soils (< 2cm) were collected from all trapping grids on both the petrochemical sites and the reference sites. Petrochemical sites were divided into six sub-sites and a composite sample was collected from each sub-site. Six subsamples were mixed to form each composite soil sample. Because there was less chemical variability, only two composite soil samples composed of six subsamples were collected from the reference site. All soils were stored and transported in sealed acid washed glass jars. Soils were air-dried and sieved to pass a 2 mm screen prior to analysis.

Soil properties (pH, organic carbon, texture, and electrical conductivity) were measured on the collected samples (Table 1). Soil pH was determined in a 1:2 soil: 0.01 M $CaCl_2$ suspension (Thomas 1996). Soil organic carbon was determined by dry combustion on a Carlo-Erba (Nelson and Sommers 1996) Soil texture was determined by the hydrometer method (Gee and Bauder 1986).

Electrical conductivity was measured in a 1:5 soil:deionized water extract (Rhoades 1996).

Metals and fluoride in soil were determined. Metals measured included Ba, Cd, Co, Cr, Cu, Ni, Pb, Sr, Ti, V, and Zn. Soil was acid digested according to U.S. EPA Method 3050 as follows. Soil (2 g) were placed in a 100 ml Teflon beaker, 10.0 ml of 8 M trace metal HNO_3 was added, and the samples were covered with a watch glass and heated on a hotplate at 95^0 C for 30 minutes. The beakers were uncovered and concentrated HNO_3 was incrementally added in 5.0 ml volumes until brown fumes were not emitted upon the addition of concentrated acid. Next, hydrogen peroxide (30%) was added in 1.0 ml aliquots with warming until the sample did not effervesce. Then, 10.0 ml of trace metal concentrated HCl was added and the temperature was raised to 150^0 C. The sample was heated for approximately 15.0 minutes, cooled, and vacuum filtered through a 0.45 Supor membrane filter. The sample was then diluted with deionized distilled water to a final volume of 50 ml. Subsequent quantitation was by inductively coupled plasma atomic emission spectroscopy (ICP-AES.)

Two forms of fluoride, total and a potentially bioavailable form, were determined in soil. Because acid extractions of soils result in low recoveries of total fluoride due to the presence of non-acid labile fluorides (Hall 1968, Cooke et al 1976, Andrews et al 1989), fusion techniques are required to accurately measure total soil F (Venkateswarlu 1983). Therefore, fusion with NaOH was used to measure total fluoride in soil (McQuaker and Gurney 1977). The fused sample was dissolved in deionized distilled water, neutralized with HCl, diluted to

a volume of 100 ml, and fluoride was determined using a combination ion selective electrode. Blanks and spike recoveries were used for quality assurance.

A weak acid extraction (0.03 M HCl, pH 1.5) followed by potentiometric determination was used to measure the potentially bioavailable fluoride (Walton 1987). As with total fluoride, blanks and spike recoveries were used for quality assurance.

Collection of Animals and Preparation of Bones

A total of 24 adult cotton rats (*Sigmodon hispidus*) were collected from each petrochemical and reference site during spring and fall trapping periods. The rats were captured with Sherman live traps placed on a 10 m spacing and baited with rolled oats. Trapping grids consisted of eight lines with eight traps per line on most sites. On some sites, modifications had to be made in the layout of traps because of physical barriers such as pits. On all sites, a total of 64 traps were utilized in the collection of animals. Following capture, the rats were housed overnight and were sacrificed the next morning by exsanguination. Two humeri from each rat were cleaned of excess tissue with a scalpel and scissors, freeze-dried, weighed, and placed in petroleum ether for 96 hours with daily changes to eliminate fat (Paranjpe et al. 1994). At the time of termination, rat skulls were removed and formalin fixed for later evaluation of incisors.

Bone Metal and Bone Fluoride Analysis

In order to measure both metals and total fluoride, each pair of bones was acid digested by a method adapted from Andrews and Cooke (1989). Each pair of bones (100 mg) was refluxed on a hotplate at 95^0C with 5.0 ml of concentrated trace metal HNO_3 for 1.0 h. Following digestion, the material was diluted with deionized distilled water to a final volume of 10.0 ml. A 1.0 ml aliquot was saved for fluoride analysis and the other 9.0 ml was analyzed for metals by ICP-AES. Metals analyzed in bone included Ba, Cr, Pb, Sr, Zn, Ti. Blanks and spike recoveries for each metal were used for quality assurance.

For bone fluoride analysis, the retained 1.0 ml aliquot of the diluted digest was diluted with deionized distilled water to a volume of 5.0 ml and subsequently combined with an equal volume of TISAB II buffer to adjust ionic strength and inhibit complexation of F by Fe and Al interferrants (Frankenberger 1996,Orion 1996). Solution pH was checked and adjusted to 5.0-5.5 by adding $\approx$ 500 µl of 10 M NaOH. Calibration standards were prepared in a similar manner from Fisher-certified 100 mg L^{-1} and fluoride concentration was determined with an Orion combination fluoride ion-selective electrode and reported as mg kg^{-1} on a freeze-dried basis. Blanks, standard reference material (NIST bone meal SRM 1486), and spike recoveries were used for quality assurance.

Scoring Of Teeth for Dental Lesions

Scoring of incisors was performed to document gross morphological lesions commonly referred to as fluorosis using a system previously described for

mammals (Boulton et al. 1994, Schupe et al. 1972). All rats were assigned a random number to prevent bias and were scored by two different analysts for confirmation (Schroder 1998).

Statistical Analysis

Soil data were analyzed as a randomized complete block design with subsampling, sites were considered blocks. PROC GLM (SAS, 1996) was used to perform the analysis of variance. The responses were transformed using the natural logarithm function to help correct for heterogeneity of variance. In order to ascertain which sites were contaminated, the means of each of the petrochemical sites were compared to the combined mean of all reference sites using Duncan's multiple range test.

Tissue data were analyzed as a split block arrangement in a randomized block design with subsampling. Sites were considered blocks and season as the split-unit treatment. PROC MIXED (SAS, 1996) was used to perform the analysis. Log transformations were used to account for heterogeneity of variance. If the treatment and season interaction was significant, an analysis of the simple effects of treatment (controlling for season) was performed using the SLICE option from an LSMEANS statement. All control sites were combined and Duncan's multiple range test was used to determine which of the petrochemical sites were contaminated. Pearson's linear correlation coefficients were calculated using PROC CORR (SAS, 1996) to evaluate the relationship between metal content in the bone of cotton rats and in the soil samples. The correlations were performed on a plot basis, taking means of the soil samples as well as the

mean of the bone samples taken from each plot. Fisher's exact test was used to analyze the relationship between bone fluoride and incisor score. Incisor scores of ≥ 3 were categorized as 'high' and < 3 as 'low'. Bone fluoride values < 1000 mg kg^{-1} were categorized as 'low', ≥ 1000 mg kg^{-1} but < 3000 mg kg^{-1} as 'medium', and values ≥ 3000 mg kg^{-1} as 'high'. A 2x3 contingency table of these two categorical variables was analyzed using PROC FREQ (SAS, 1996). A chi-square test of independence was performed to test whether the percentage of high incisor scores were equal for the cotton rats in the three bone fluoride categories.

Results and Discussion

Soils

Analysis of variance found metal concentrations in soil was elevated on the petrochemical sites as compared to the reference sites for several metals including: Cd ($p = 0.016$), Cr ($p = 0.003$), Cu ($p = 0.002$), Ni ($p = 0.005$), Pb ($p = 0.0002$), Sr ($p = 0.006.$), Ti ($p = 0.025$), V ($p = 0.018$), and Zn ($p = 0.0001$). The mean total soil content for all the metals except Ti on the reference sites were similar to values reported for uncontaminated sites (Table 2). Titanium content of baseline soils reported by Kabata-Pendias and Pendias (1984) were summarized from studies that used wet digestion of soil with HF. Most soil Ti occurs as TiO_2 which is only dissolved by using acid digestion with HF. Soil Ti from petrochemical and reference sites in our study were determined by a wet chemical digestion (USEPA Method 3050) that does not incorporate HF but is

designed to measure anthropogenic metals. Therefore, soil Ti values measured in our study are lower than soil Ti levels measured by HF digestion. Duncan's multiple range test indicated that the number of sites with elevated levels varied between metals. The number of sites on which the metal level was elevated (in parenthesis) as compared to the mean of all the reference sites was Ba (3), Cd (2), Co (3), Cr (9), Cu (8), Ni (7), Pb (9), Sr (6), Ti (5), V (5), and Zn (12) (Tables 3-4). The predominant elevated metals in soils on the petrochemical sites were Cr, Cu, Ni, Pb, Sr, and Zn. Elevated levels of Cr in soil ranged from 2-fold to more than 100-fold greater than the overall mean of the reference sites. Elevated levels of Cu in soil were 2- to 85-fold greater than the overall mean of the reference sites. Elevated levels of Ni in soil were 1.5- to 3-fold greater than the overall mean of the reference sites. Elevated levels of Pb in soil were 5- to 140-fold greater than the overall mean of the reference sites. Elevated levels of Sr in soil were 2- to 20-fold greater than the overall mean of the reference sites. Elevated levels of Zn in soil were 2- to 26-fold greater than the overall mean of the reference sites. Although the sites were classified as landfarms, pond burms, and tar pits; metal contamination was randomly distributed among these three classifications. Loehr (1993) examined a land treatment unit to which petrochemical waste had been applied for over 30 years. Metals that were elevated on his study site were Cr (mean of 280 mg kg^{-1}), Pb (mean of 130 mg kg^{-1}), Ni (mean of 110 mg kg^{-1}), and Zn (mean of 235 mg kg^{-1}). Several of the sites examined in our study have similar or lower levels of Cr, Ni, and Zn (Tables 3-4). However, there are several sites in our study, which have much higher

levels of Pb than was reported by Loehr (1993).

Both the total fluoride in soil ($p = 0.001$) and HCl extractable form of fluoride ($p = 0.002$) were elevated on the petrochemical sites as compared to the reference sites. The total content of fluoride in the soil of reference sites was similar to levels from uncontaminated sites which ranges from 10 to 400 mg kg^{-1} depending on soil texture (Table 2). Total fluoride was elevated on seven of the sites and the HCl extractable form of fluoride was elevated on nine of the petrochemical sites (Table 4). The HCl extractable form of fluoride was 4- to 25-fold greater on the elevated sites as compared to the overall mean of the reference sites. Total fluoride was 10- to 60-fold greater on the elevated sites as compared to the overall mean of the reference site. In a detailed investigation of a landfarm, Schroder et al. (1998) found elevated levels of both total fluoride (mean of 1954 mg kg^{-1}) and HCl extractable fluoride (mean of 326 mg kg^{-1}) in soil to which oily-sludges containing HF had been applied. The results of this study are consistent with their findings in that all five of the landfarms (sites A through E) had elevated levels of both of these forms of fluoride. Therefore, it does appear that fluoride in soil is more prevalent on landfarms than on the other types of petrochemical sites.

Bone Metal and Fluoride Content

The overall mean content of Pb in bone was elevated ($p = 0.003$) for cotton rats collected from the petrochemical sites as compared to the reference sites. There was a significant interaction of treatment and season for Pb content

(p = 0 .0175) in cotton rat bone. Analysis using the SLICE option of the LSMEANS statement showed that that Pb levels in bone of 21.5 mg kg^{-1} were higher in cotton rats collected from the petrochemical sites in winter as compared to Pb content of 10.0 mg kg^{-1} in bone of animals collected during the summer (p = 0.0003). Levels of lead in bone tissue of mice and moles collected from uncontaminated sites typically range from 2-3 mg kg^{-1} or lower (Ma 1996). Pb concentrations in bone of cotton rats collected from the reference sites of 1.5 mg kg^{-1} were similar to these levels. Duncan's multiple range test indicated that the number of sites with elevated levels of metal in cotton rats varied between metals. The number of sites on which the metal level was elevated (in parenthesis) as compared to the mean of all the reference sites was Ba (1), Cr (6), Pb (8), Sr (4), Ti (0), and Zn (1) (Table 5). Of the metals examined; Cr, Pb, and Sr were the most prevalent in bone tissue of cotton rats collected from the petrochemical sites. Cr content of bone were slightly elevated on some sites and were approximately 2-fold greater than the overall mean of bone Cr in cotton rats collected from the reference sites. Although there is very limited data available on wildlife studies, Cr content in bone of cotton rats collected from the reference sites were similar to levels reported in other small mammal studies on uncontaminated sites which may range from 0.1 to 10 mg kg^{-1} (Outridge and Schuehammer 1993). While several studies have documented the primary organs associated with bioaccumulation Cr in small mammals as being the kidneys and liver, Taylor and Parr (1978) examined cotton rats collected from downwind of an airborne Cr source and found that the majority of Cr was present

in bone tissue. Cotton rats examined in their study contained 0.46 mg kg^{-1} on the polluted area as compared to a value of 0.16 mg kg^{-1} on their reference site. The results of our study are much higher than their findings. However, the concentrations documented in our study are considerably < 4 mg kg^{-1} Eisler (1986) considered to be indicative of likely contamination by Cr. The significance of elevated Cr in tissues of wildlife is unknown, because of extremely limited toxicological data on wildlife (Outridge and Schuehammer 1993). The elevated concentrations of Pb in bone were approximately 2- to 42-fold greater than the overall mean of cotton rats collected from the reference sites. Pb is not an essential element in mammalian systems and chronic exposure to Pb may result in renal dysfunction, reduced growth rate, and reproductive impairment (Tsuchiya 1986, Venugopal and Luckey 1978). Most Pb usually enters small mammals through ingestion and greater than 90% of the lead in small mammals is found in bone tissue (Talmage and Walton 1991). Several studies have examined bioaccumulation of Pb in small mammals associated with elevated levels of Pb in soil. These studies range from studies done on highly contaminated smelter areas to earlier air pollution studies on less contaminated urban areas. Johnson et al. (1978) examined soils and wood mice (*Apodemus sylvaticus*) collected from a lead-zinc mining site. Pb was elevated in both soil (mean of 8430 mg kg^{-1}) and in bone of wood mice (mean of 352 mg kg^{-1}). In another study, Roberts et al. (1978) found elevated Pb (mean of 189 mg kg^{-1}) in bone of wood mice (*Apodemus sylvaticus*) collected from a lead-zinc mining site with elevated Pb in soil (mean of 14,010 mg kg^{-1}). Animals collected in both of these studies

displayed intranuclear inclusion bodies in the kidneys , a classic and sensitive indicator of lead poisoning. In general, Pb levels in cotton rats from the petrochemical sites are much lower than other small mammals collected from sites contaminated by metal mining and smelting.

The elevated concentrations of Sr in bone were only slightly elevated and were approximately 1.5-fold greater than the overall mean of cotton rats collected from the reference sites.

The overall mean content of fluoride in bone was elevated ($p = 0.004$) for cotton rats collected from the petrochemical sites as compared to the reference sites. There was a significant interaction of treatment and season for fluoride content ($p = 0 .0377$) in cotton rat bone. Analysis using the SLICE option of the LSMEANS statement showed that that fluoride levels of 1926 mg kg^{-1} in bone were higher in cotton rats collected from the petrochemical sites in winter as compared to fluoride content of 788 mg kg^{-1} in bone of animals collected during the summer ($p = 0.0001$). Fluoride concentrations in bone of cotton rats collected from the reference sites were similar to levels reported in other small mammal studies on uncontaminated sites. Concentrations of fluoride in bone for several species of small mammals collected on an uncontaminated site ranged from 133-144 mg kg^{-1} (Kay et al. 1975). Fluoride content of bone was also elevated on seven of the petrochemical sites as compared to the overall mean of the reference sites (Table 5). Elevated fluoride concentrations in bone were approximately 5- to 23-fold greater than the overall mean of cotton rats collected from the reference sites. Elevated levels of fluoride in bone of small mammals

have been associated with elevated levels of soil fluoride on numerous sites (Andrews et al. 1982, Andrews et al. 1989, Wright et al. 1978). The results of this study are similar to those of Schroder et al. (1998) who reported elevated levels of fluoride in bone (mean of 1515 mg kg^{-1}) of cotton rats collected from a landfarm to which oily-sludges that contained HF had been applied.

Dental Lesions

Cotton rats and other herbivorous small mammals depend upon their teeth for preparation of food before digestion. Dental lesions have been noted for various species of small mammals collected from fluoride contaminated environments. In two different studies in Europe, high bone fluoride and dental lesions were found in rodents and shrews (Walton 1986a, Walton 1986b). An incisor scoring system has been used to evaluate the degree of fluorosis in deer (Kierdorf et al. 1996) and voles (Boulton 1994). Additionally, Boulton (1995) employed an incisor scoring system to evaluate various small mammals that had been dosed with fluoride. Gross dental lesions in teeth of small mammals have been used as an indicator of fluorosis and may affect feeding behavior of such animals (Cooke et al. 1996). Schroder et al. (1998) reported that ≈80% of the cotton rats collected from a landfarm contaminated with fluoride exhibited dental lesions (severity score ≥ 3). The prevalence of dental fluorosis in this study was somewhat less in that approximately 50% of the cotton rats captured on the seven petrochemical sites with elevated levels of soil and bone fluoride displayed dental lesions (severity score ≥ 3). The majority (> 99%) of the cotton rats collected from the reference sites in this study did not have dental lesions.

Severity of dental lesions varied from site to site and ranged from a score of one (slight striation in lower incisor) to a score of five (white chalky lower and upper incisors). Overall approximately 80% of the cotton rats collected from the seven petrochemical sites with elevated levels of soil and bone fluoride had some form of dental lesions (severity score of 1 to 5). The prevalence of dental fluorosis was approximately 50% higher in winter than in summer animals. Dental lesions were more prevalent on sites A, C, D, and L than on the other sites. However, more than 50% of the cotton rats collected from sites B, E, and H had lesions (Figure 1). Regression analysis revealed a strong relationship ($p = 0.0001$) between incisor score and fluoride content in bone of cotton rats. However, a more detailed analysis using Fisher's exact test indicated that the severity of dental fluorosis could not always be accurately predicted by the concentration of fluoride in bone. By classifying total content of fluoride in bone as low (<1000 mg kg^{-1}), medium ($\geq$ 1000 but < 3000 mg kg^{-1}), or high ($\geq$ 3000 mg kg^{-1}) and ranking dental lesions in cotton rats as low (< 3) or high ($\geq$ 3), it was possible to determine whether fluoride content in bone could predict the severity of dental fluorosis in cotton rats. The analysis revealed that only 5% of the cotton rats had a high severity score when bone fluoride concentrations are less than 1000 mg kg^{-1}. Thus, low levels of bone fluoride can accurately predict the severity of dental fluorosis. Approximately 52% of the animals collected had a high severity score when bone fluoride ranged from 1000 to 3000 mg kg^{-1}. Medium levels of fluoride in bone could not be used to predict the severity of dental fluorosis. At bone fluoride levels greater than 3000 mg kg^{-1}, approximately 78% of the rats

had a high severity score. Therefore, high levels of bone fluoride can accurately predict the severity of dental fluorosis

Relationship Between Bone and Soil Concentrations

Although elevated levels of metal were found in both soils and cotton rats from the petrochemical sites, there is not a strong relationship between metal content of bone and soil metal concentrations. (Table 6). However, there is a strong relationship between bone fluoride and HCl extractable fluoride and total forms of fluoride in soil.

Elevated levels of Cd and Pb in tissue of wood mice (*Apodemus sylvaticus*) have been associated with elevated concentrations of Cd and Pb in soil on smelter and mining sites (Talmage and Walton 1991). Shore et al. (1995) examined published studies on small mammals and soil concentrations of Pb and fluoride. Their study indicated a positive relationship between soil Pb and Pb content in bone of wood mice ($r = 0.714$) and field voles ($r = 1.000$). There was insufficient data available for Talmage and Walton (1991) to show a relationship between soil fluoride and bone fluoride. However, Scharma and Shupe (1977) did not find a significant between Pb levels in soil and tissue concentrations in rock squirrels (*Spermophilus varigatus*). Their study concentrated on a much narrower range of soil concentrations of Pb as compared to the study by Talmage and Walton (1991). Similarly, the soil Pb in our study covers a much smaller range and overall is considerably lower than the Pb levels examined by Talmage and Walton (1991). Perhaps relationships between soil concentrations

of Pb and bone Pb may be difficult to determine when relatively small ranges of soil contamination are examined.

Conclusion

Petrochemical waste disposal resulted in contamination of soil with Cd, Cr, Cu, Ni, Pb, Sr, Ti, V, Zn, and F. Contamination is more prevalent for F on landfarms vs. other waste disposal (tar pits, etc.) areas. However, metal contamination appears to be randomly distributed among landfarms, pond burms, and tar pits. Elevated levels of Cr, Pb, Sr, and F were found in bone tissue of cotton rats collected from some of the petrochemical sites. Pb and fluoride were the most common contaminants found on cotton rat bones and fluoride bioaccumulation was more prevalent on landfarms than on other areas. Additionally, Pb and fluoride levels in bone of cotton rats collected during winter were higher than in cotton rats collected during the summer. Effects from Cr, Pb, and Sr were not evident but cotton rats collected from the sites with elevated soil and bone fluoride displayed dental lesions which is a classic sign of fluorosis. The prevalence of dental fluorosis was seasonally dependent and was approximately 50% higher in winter than in summer animals.

Although soil is a likely source of metal contamination, the relationship between concentration of metals in soil and bone was poor. Perhaps the levels in our study were too low to establish this relationship. However, there is a strong relationship between bone fluoride and HCl extractable fluoride and total content of fluoride in soil.

Disposal of petrochemical waste may result in elevated level of inorganic contaminants that may pose a threat to terrestrial organisms. Therefore, to prevent accumulation of contaminants in cotton rats, petrochemical wastes should be monitored for inorganic contaminants and land application rates should be based on the level of inorganic contaminants. Waste that contains excessive levels of inorganic contaminants may not be suitable for land application.

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Table 1. Description of petrochemical contaminated soils.

Site	Type	Soil pH	Soil OC[a]	Soil Texture	Soil EC[b]
A	landfarm	7.5	3.2	loam	0.24
B	landfarm	6.6	4.7	loam	0.21
C	landfarm	6.5	4.7	loam	0.19
D	landfarm	7.0	6.5	sandy loam	0.27
E	landfarm	6.9	14.5	sandy loam	0.32
F	pond burm	7.1	7.9	loam	0.20
G	pond burm	6.8	3.9	loam	0.16
H	pond burm	5.1	33.8	loamy sand	0.18
I	tar pit	6.0	3.3	silt loam	0.13
J	tar pit	7.0	3.4	loam	0.23
K	tar pit	6.6	3.4	clay loam	0.21
L	tar pit	6.5	30.4	sandy loam	0.18

[a]organic carbon content in %
[b]electrical conductivity (dS m^{-1})

Table 2. Comparison of range and mean metal content of study site with baseline soils.

Metal	Petroleum sites[a]	Reference sites	Baseline soils
Ba	83-312 (211)	16.0-883 (196)	100-3000[b] (580)
Cd	0.10-5.12 (0.96)	0.00-0.60 (0.25)	0.00-0.61[c] (0.22)
Co	3.78-12.30 (8.82)	3.6-17.5 (7.94)	6.3-30.3[c] (14.0)
Cr	7.70-1863 (267)	3.9-52.6 (18.3)	5.0-1500[b] (54.0)
Cu	16.8-1210 (152)	5.3-74.0 (14.2)	2.7-23.9[c] (10.5)
Ni	12.4-50.6 (29.2)	5.8-28.6 (15.5)	6.1-41.7[c] (21.0)
Pb	20.9-1679 (410)	4.1-29.8 (12.0)	5.1-27.2[c] (16.5)
Sr	16.7-390 (86.3)	9.2-47.6 (18.2)	10.0-500[b] (67.0)
Ti	9.23-223 (73.0)	5.4-228 (51.3)	684-4081[c] (2765)
V	11.8-95.7 (42.8)	4.9-50.7 (21.2)	3.8-81.0[c] (31.7)
Zn	58.3-894 (208)	12.9-51.6 (34.9)	22.3-127.3[c] (31.7)
Hcl F	2.0-1026 (247)	0.6-26.5 (4.03)	----------
Fusion F	60.2-5257 (1748)	10.9-217 (89.7)	10.0-400[d] (360)

[a] Range and mean (in parenthesis) metal content of soils
[b] Adriano 1986
[c] Basta et al. 1998
[d] Kabata-Pendias and Pendias 1984

Table 3. Total mean concentrations of metals and fluoride in soils from petrochemical sites. All values are in mg kg^{-1} on a soil basis. Bolded values are greater ($p < 0.05$) than the mean of all reference sites. Values with the same letter are not significantly different.

Site	Ba	Cd	Co	Cr	Cu	Ni	Pb
a	206 bcd	0.48 bcd	6.82 de	**233 b**	**36.5 cde**	**50.6 ab**	**61.1 ef**
b	193 bcd	0.32 bcd	**17.8 a**	**52.8 de**	18.5 fg	**31.1 abcd**	20.9 h
c	160 bcdef	0.38 bcd	**11.2 bc**	**105 c**	24.8 defg	19.6 bcdef	29.1 fgh
d	**273 abc**	0.33 bcd	9.80 bcd	**292 b**	**102 b**	**27.7 abcd**	**1240 a**
e	**312 ab**	**2.38 a**	7.30 de	**1863 a**	**1210 a**	**38.7 a**	**1679 a**
f	169 cdef	0.48 bcd	3.78 g	**423 b**	**195 b**	12.4 f	**769 b**
g	191 bcde	0.73 b	9.78 bcd	7.7 i	16.8 fg	14.9 def	**343 bc**
h	161 cdef	0.23 cd	7.43 de	**95.9 cd**	**54.4 cd**	**35.8 ab**	**243 bcd**
I	212 bcdef	0.32 bcd	4.68 fg	13.1 hi	**51.0 cd**	19.8 cdef	24.2 gh
j	82.9 f	0.70 bc	8.49 cde	26.3 fg	**68.9 bc**	26.3 abcde	**170 de**
k	**483 a**	**5.12 a**	**12.3 b**	**37.5 ef**	18.1 efg	**32.0 abc**	**147 efg**
l	87.4 ef	0.10 d	6.45 ef	**54.0 de**	**30.3 cdef**	**42.0 ab**	**198 cde**
Reference	196 def	0.25 bcd	7.94 de	18.3 gh	14.2 g	15.5 ef	12.0 h

Table 4. Total mean concentrations of metals and fluoride in soils from petrochemical sites. All values are in mg kg^{-1} on a soil basis. Bolded values are greater ($p < 0.05$) than the mean of all reference sites. Values with the same letter are not significantly different.

Site	Sr	Ti	V	Zn	HCl F	Fusion F
A	**192 b**	**164 ab**	**92.4 a**	**173 bc**	**732 a**	**2672 bc**
B	19.2 f	19.2 f	23.0 c	**90.9 de**	**338 b**	**878 d**
C	23.5 def	23.5 ef	23.1 c	**259 b**	**1026 a**	**4316 ab**
D	**74.8 c**	**124 abc**	**70.2 ab**	**215 b**	**344 b**	**5257 a**
E	**390 a**	**223 a**	**40.8 b**	**894 a**	**22.2 d**	**2082 bc**
F	**158 c**	31.0 ef	14.0 de	**83.8 de**	**20.5 de**	64.7 g
G	25.1 def	25.1 ef	8.1 e	**249 b**	6.23 fg	103 efg
H	**50.3 d**	**104 bc**	**93.9 a**	**96.3 de**	**124 c**	**3213 bc**
I	**37.6 de**	50.8 de	11.8 de	**87.8 de**	2.04h	60.2 fg
J	16.7 f	9.23 g	17.1 cd	**140 cd**	**16.1 ef**	169 e
K	26.8 def	26.8 ef	22.9 c	**153 de**	4.37 gh	150 ef
L	21.3 ef	**74.9 cd**	**95.7 a**	**58.3 e**	**332 b**	**2016 cd**
Reference	18.2 f	51.3 ef	21.2 cd	34.9 f	4.03 gh	89.7 efg

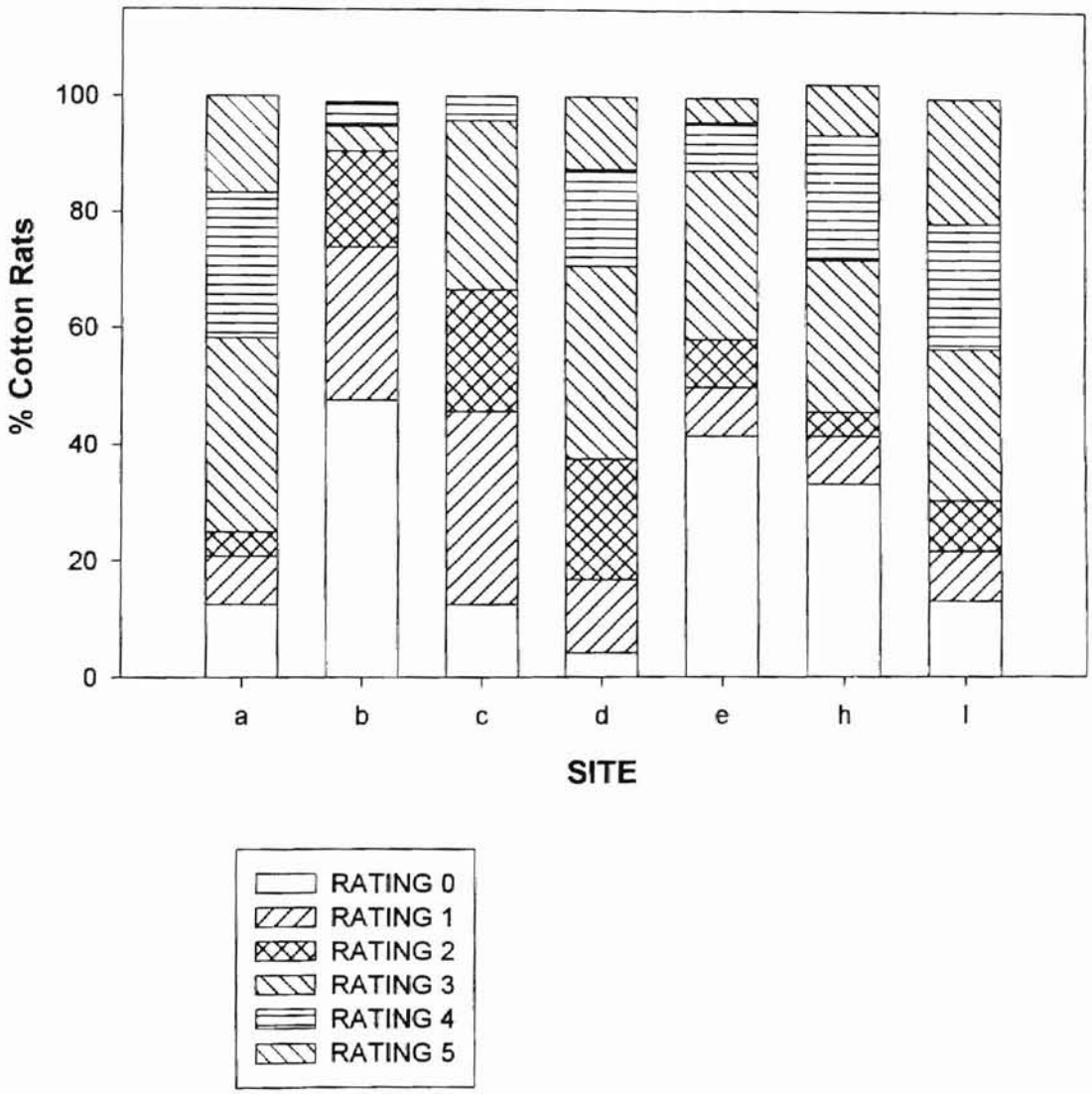
Table 5. Mean concentration of bone in cotton rats collected from petrochemical sites. All values are in mg kg^{-1} of bone. Bolded values are greater ($p<0.05$) than the control. Values with the same letter are not significantly different

Site	Ba	Cr	Pb	Sr	Ti	Zn	F
A	29.5 ef	**2.9 b**	**4.6 c**	**239 ab**	0.5 a	179 b	**1515 bc**
B	45.6 cd	1.4 cd	1.4 def	134 e	0.3 ab	184 ab	**1610 bc**
C	40.2 de	0.5 d	0.7 f	133 e	0.2 b	177 b	**2964 a**
D	65.5 bc	**2.9 ab**	**63.4 a**	145 de	0.3 ab	185 ab	**830d**
E	61.9 bc	**3.2 a**	**12.8 b**	**174 cd**	0.3 ab	167 bc	**1733 c**
F	31.1 ef	0.4 d	**12.4 b**	**212 bc**	0.5 ab	170 bc	89.5 f
G	47.2 cd	0.8 cd	**60.7 a**	132 e	0.4 ab	180 b	171 e
H	79.4 b	**2.7 ab**	2.2 def	134 e	0.3 ab	172 bc	**2671 b**
I	81.5 b	0.7 d	**3.5 cd**	**257 a**	0.5 ab	150 c	137 e
J	21.3 f	**3.7 ab**	**3.8 c**	83.5 f	0.4 ab	163 bc	172.6 e
K	**126 a**	1.3 cd	3.0 cde	163 cde	0.2 b	211 a	137.5 e
L	78.4 b	**2.9 ab**	**20.1 b**	134 e	0.3 ab	197 b	**3683a**
Reference	105 b	1.6c	1.5 ef	148 e	0.4ab	173 bc	159 e

Table 6. Simple correlation between bone and soil contents.

	Ba	Cr	Pb	Sr	Ti	Zn	HCl F	Total F
r	-0.00	0.30	0.36	0.40	0.05	-0.07	0.70	0.85
p-value	1.00	0.34	0.25	0.21	0.89	0.84	0.02	0.00

Fig. 1. Severity of fluoride-induced lesions in incisors of cotton rats captured from petrochemical sites



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